Bacterial export takes its Tol

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The recent crystal structure of TolC elegantly indicates its function and provides insight into its mechanism for export of a wide range of molecules across the periplasmic space and outer membrane of Gram-negative bacteria. The structure is compared to those of other proteins that are embedded in bacterial outer membranes or that traverse the periplasmic space.

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Introduction

Active-transport across biological membranes utilizes energy transduction. Typically, one or more components of the electrochemical gradient of a membrane drive structural changes in a transporter localized within that membrane. These conformational changes form a transport cycle enabling the uptake or efflux of substrate. Another category of transporters, ABC (ATP-binding cassette) transporters, utilizes enzymatic hydrolysis of ATP to effect structural changes for transport; this class of transporters is beyond the scope of this short review. The outer membranes of Gram-negative bacteria lack a local electrochemical gradient and therefore present special challenges to this paradigm of transport processes. Passive or facilitated diffusion through porins renders the outer membrane permeable to molecules of approximate molecular weight ≤ 1000 [1]. However, bacteria import or export much larger molecules across the outer membrane. This process of transport across the outer membrane, periplasmic space and inner membrane is carried out by a variety of multiprotein systems. The task of each of these systems is twofold: binding and transport of a substrate or ligand across each of these three components of the cell; and coupling the inner membrane electrochemical gradient to facilitate active-transport across the outer membrane. The TolC system uses an inner-membrane translocase and an outer-membrane protein, TolC, to export a variety of molecules ranging from antibiotics to bacterial toxins of over 100 kDa in mass [2–4]. In addition to its fundamental interest, the TolC system is likely to have a role in bacterial multidrug resistance and pathogenicity.

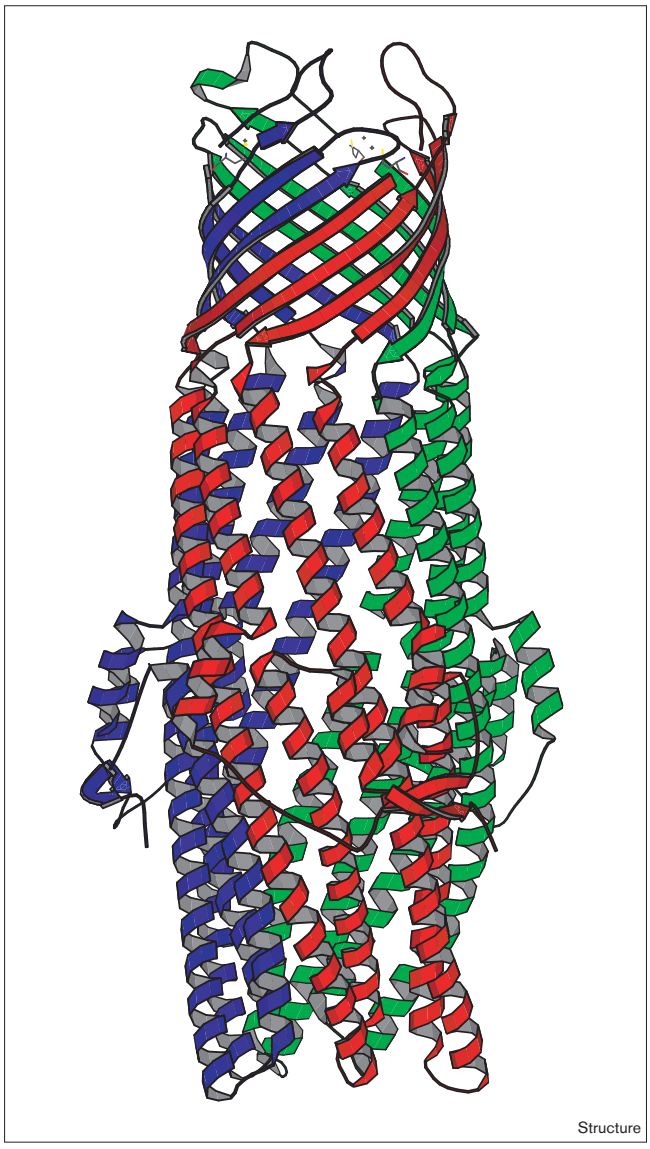
A beautiful structure indeed

Structural biology, particularly X-ray crystallography, is a thriving and expanding scientific discipline. The Protein Data Bank contains over 12,000 entries with, on average, more than seven depositions made per day in 1999. Structures permeate much of the biochemical and biomedical sciences, and it is easy for both layperson and cognoscente alike to be somewhat jaded about the appearance of new structures. However, on rare occasion, a protein structure illuminates and informs function and mechanism in a manner that is direct, clear and, frankly, a joy to behold. Consider the structure of TolC to be in this category. Previous studies [2,3] indicated that TolC forms a channel coupling an inner-membrane translocase to a pore in the outer membrane. The structure of TolC reveals that it is both the channel and the pore (Figure 1). A trimer of TolC molecules forms the assembly, and it appears that this assembly is the functional channel/pore unit. There are three domains in each TolC monomer. Using the nomenclature of Koronakis *et al.* [4], each TolC monomer contributes four β strands (the β domain containing strands S1–S4) to form a 12-stranded β barrel; the height of this barrel (~40 Å) is comparable to other membrane proteins that span the outer membrane. The α-helical domain contains six α helices: two long helices (H3 and H7) each over 100 Å in length and four shorter helices (H2, H4, H6 and H8). Two pairs of these shorter helices (H2 and H4; H6 and H8) are each contiguous and coaxial in the structure, and essentially form another pair of long helices. Inserted between these pairs of helices is the mixed α/β domain (S3, S6, H1, H5 and H9). This domain juts out from the side of the α-helical domain, in a region close to the middle of the helices.

Opening and closing the channel

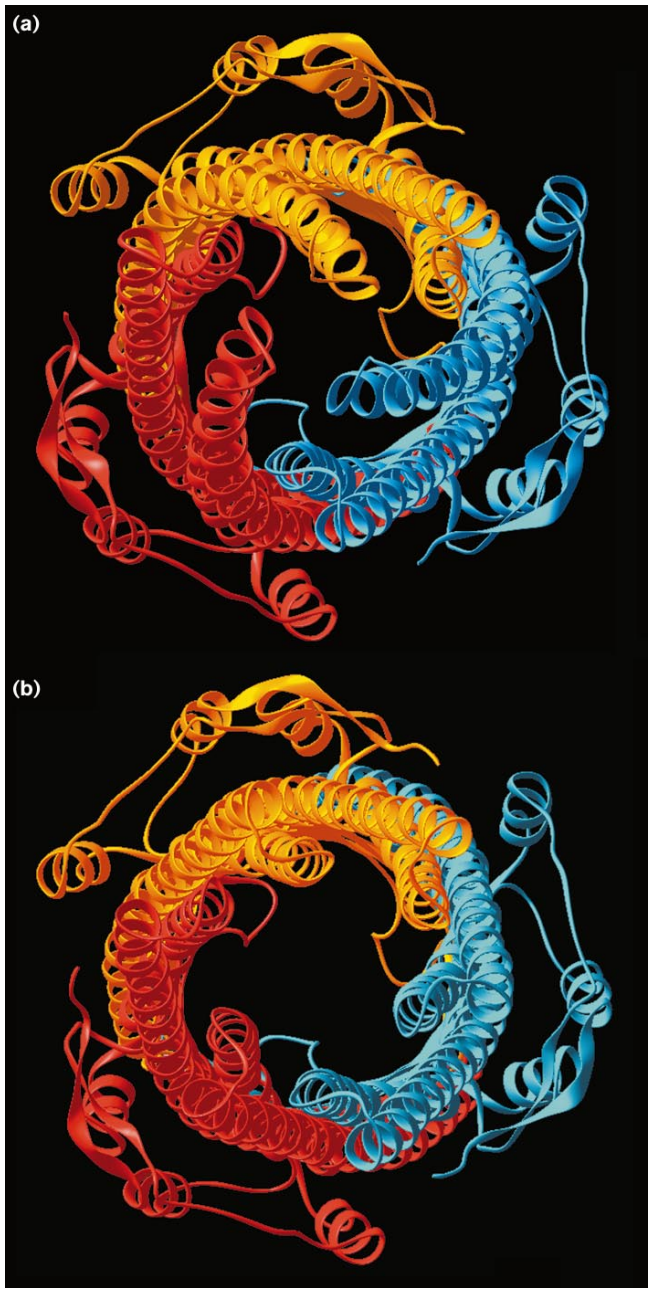
The TolC trimer is a concatenation of a 12-stranded β barrel of ~40 Å height and, as described above, a 12-helix bundle of ~100 Å height. As Koronakis *et al.* [4] note, the internal volume of this assembly is ~43,000 $Å^3$, one of the largest seen in a protein structure. However, the end of the α-helical bundle (the channel opening that is closest to the inner membrane) is closed. It thus appears, as has been the case in the structures of several bacterial channels (e.g. a potassium channel KcsA [5] and a mechanosensitive channel MscL [6]), that the structure is of the closed form. The authors propose a model for the open state of TolC (Figure 2) based upon internal structural symmetries of the individual subunits. Sequence similarity between pairs of helices in the α-helical domain indicates that similar knobs-in-holes packing could be obtained both between the 'inner pairs' of helices (seen in the closed form of the crystal structure), and in a posited open conformation where rotation of the subunits causes the 'outer pairs' to pack. This cooperative pivoting motion could open the aperture of the helical tunnel by as much

Figure 1



Ribbon diagram of the crystal structure of TolC determined by Koronakis *et al.* [4]. A trimer of TolC molecules forms the combined β barrel/α-helical 'tunnel' structure. Each monomer is shown in a different colour. This figure was made using MOLSCRIPT [19].

Figure 2



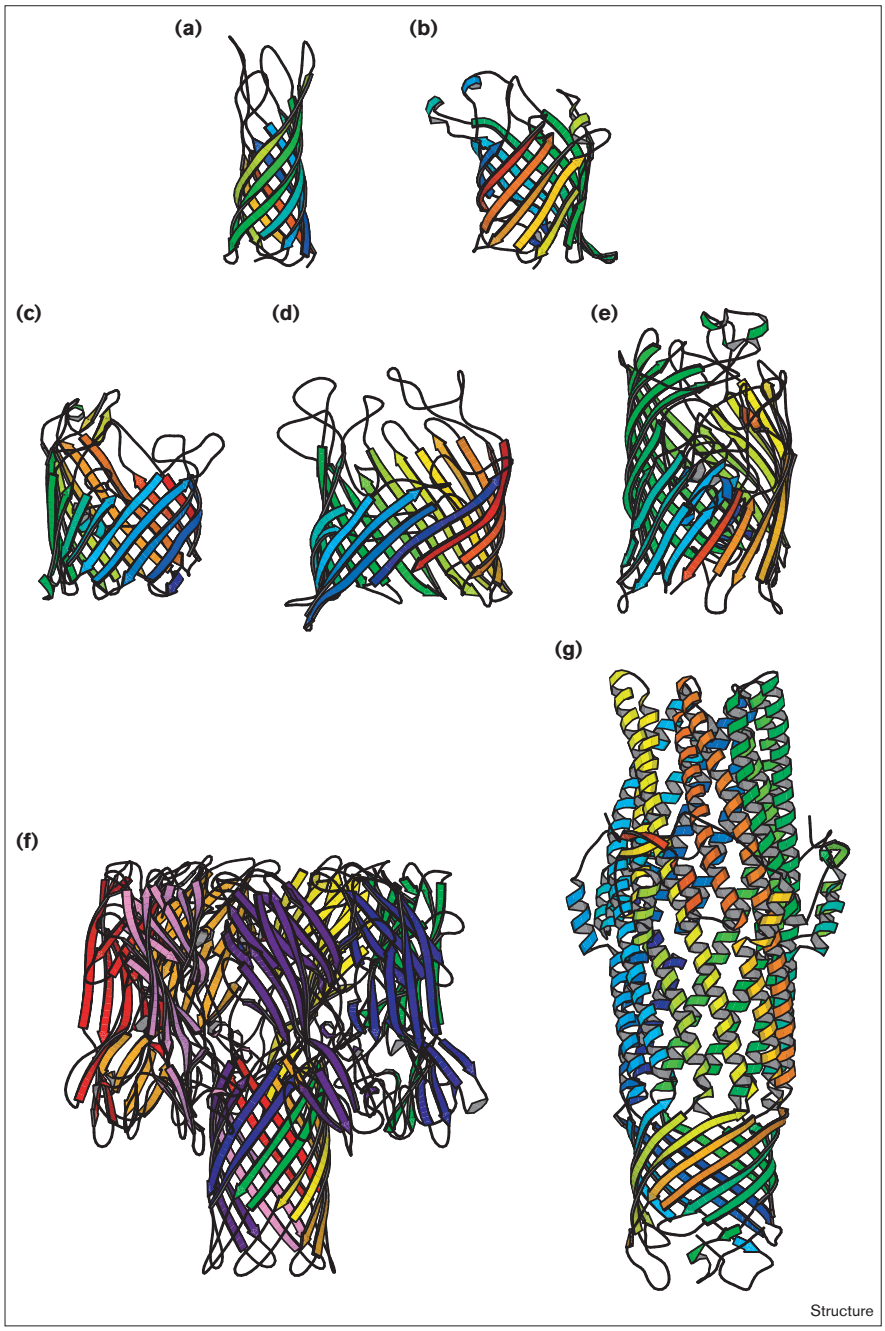
Closed and open forms of TolC. **(a)** An exterior view of the α-helical barrel as determined from the crystal structure. **(b)** A model proposed [4] for a possible conformation of the open form. The open form is achieved by a rotation of the helices that preserves nearly identical helical packing to that present in the closed form. (The figure was reproduced from [4] with permission.)

as 30 Å. At this time, this interesting structural hypothesis is neither buttressed nor weakened by experimental data. The combination of site-directed spin-labeling and electron paramagnetic resonance spectroscopy has been very powerful in elucidating structural changes during channel opening [7] and transporter–substrate interactions [8]. TolC might be another system for which this method will prove to be well suited.

Proteins in the outer membrane: the more, the beta?

To date, the crystal structures of more than ten different outer-membrane proteins have been determined. All of these proteins are β barrels, and variation of the architecture occurs in several ways (Figure 3). Different outer membrane protein structures reveal differing oligomeric states (monomeric, dimeric or trimeric) and differing numbers of β strands comprising the barrel (8, 12, 16, 18 or 22). These

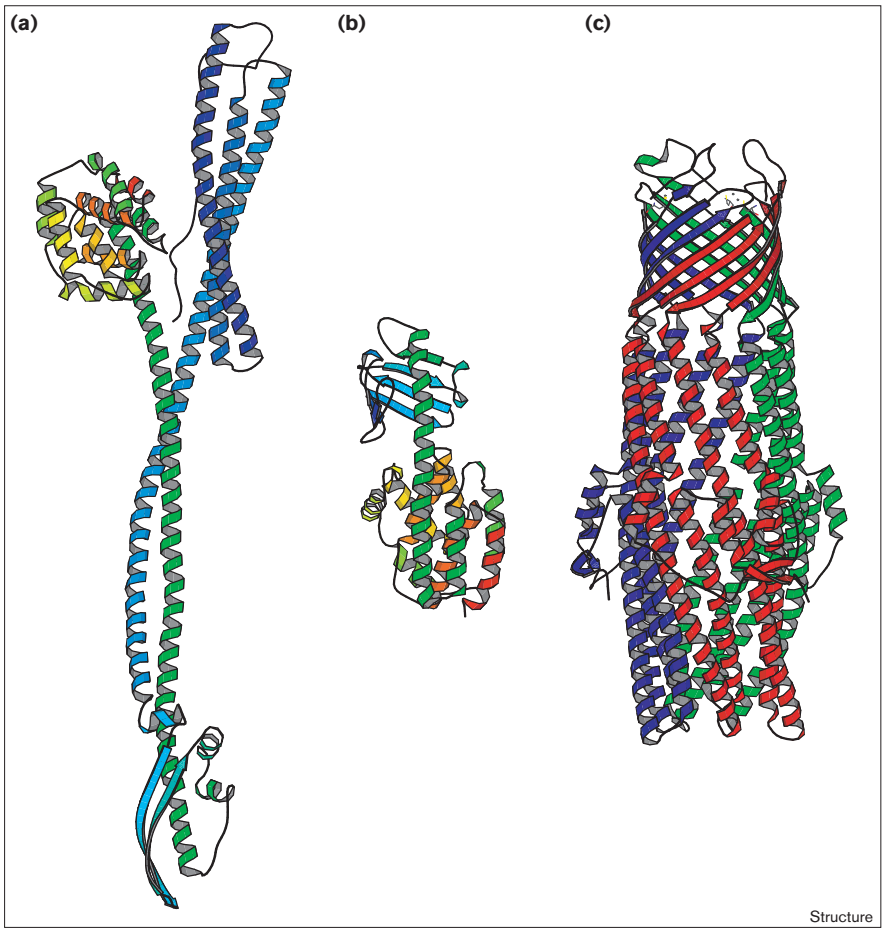
Figure 3



A bestiary of β barrels. The structures of seven representative outer-membrane proteins. Each structure is denoted by name, PDB accession number, reference, number of β strands comprising the barrel, and number of barrels comprising the observed functional oligomeric state. **(a)** OmpA, 1BXW [20], eight strands, monomer; **(b)** OMPLA, 1QD5 and 1QD6 [21], 12 strands, monomer and dimer; **(c)** OmpF, 2OMF [22], 16 strands, trimer; **(d)** LamB, 1MAL [23], 18 strands, trimer; **(e)** FhuA, 1BY5 [12] and 2FCP [13], 22 strands, monomer; **(f)** α-hemolysin, 7AHL [11], 14 strands with two strands from each of the seven polypeptide chains that comprise the barrel, monomer; **(g)** TolC, 1EK9 [4], 12 strands with four strands from each of the three polypeptide chains that comprise the barrel, monomer. Note that additional structural variations can occur among β barrels that possess the same strand number and same oligomeric state. For instance, OmpA [20] and OmpX [24] are both monomeric and eight-stranded, but possess different shear numbers [9,10]. This figure was made using MOLSCRIPT [19].

proteins also exhibit variation in the shear number, a descriptor of the tilt of β strands with respect to the axis of the barrel [9,10]. The structure of TolC differs in a fundamental way from all of the previously determined structures of outer-membrane proteins. Unlike all of these proteins where each β barrel is composed of a single polypeptide chain, the β barrel of TolC consists of four β strands from each of three subunits. This tripartite structure of the barrel of TolC is reminiscent of the channel-forming portion of the toxin α-hemolysin [11]. In this structure, the membrane-interacting portion of the structure consists of a heptamer with each subunit contributing two strands to the barrel. As a heptamer, the toxin exists in two membrane-bound forms: a prepore that is not conductive, and a pore that forms a lytic channel with a diameter of > 10 Å [11]. Perhaps the requisite conformational flexibility required by α-hemolysin (and by inference TolC) arises from multiple subunits forming a

Figure 4



A comparison of three periplasmic-spanning proteins. **(a)** Colicin Ia (PDB code 1CII [16]), **(b)** colicin N (1A87 [17]), and **(c)** TolC (1EK9 [4]). This figure was made using MOLSCRIPT [19].

single complex. An interesting contrast is seen in the structures of FhuA [12,13] and FepA [14], bacterial outer-membrane transporters that bind and transport iron-siderophore complexes across the outer membrane in an energy-dependent fashion that utilizes the TonB system. These 22-stranded β barrels are monomeric, and conformational changes during ligand binding and transport probably occur by movement or displacement of an N-terminal 'hatch' domain that occludes nearly all of the barrel in each of the crystal structures. These proteins might present a case where rigidity of the β barrel formed by a single polypeptide chain prevents large structural changes within that barrel. It is my opinion that the interesting question of why the membrane-spanning portions of outer-membrane proteins are comprised entirely of β strands, and conversely why transmembrane portions of inner (plasma) membrane proteins are all α-helical, has not yet been satisfactorily answered.

Spanning the periplasm in a single bound

The α-helical tunnel in the TolC trimer is ~100 Å in length, and is postulated to be able to span the periplasmic space. There are a range of estimates of the thickness of the periplasmic space, and there is also the possibility of contact sites between inner and outer membranes where the two are in close apposition. The uncertainty and possible fluctuation of this distance, however, in no way detracts from the likelihood of TolC spanning the periplasmic space, or at least spanning a large portion of it and being able to bind to the inner-membrane translocase involved in the export pathway. In the proposed mechanism, interaction of TolC with this translocase drives a conformational change that opens the α-helical tunnel and permits efflux. TolC is certainly not the only protein that appears to span the periplasm (or at least much of it) in order to function. Channel-forming colicins [15] are bacteriocins that bind to sensitive cells, translocate across the outer membrane (either through or assisted in some way by outer membrane proteins), bind to the inner membrane, and form lethal pores. Biochemical evidence suggests that some of these colicins contact both the inner and outer membranes. Crystal structures of the soluble forms of intact colicin Ia [16] and nearly intact colicin N [17] possess helical segments that are likely to span the

periplasm (Figure 4). In the case of colicin Ia, this is clearly seen as the helices connecting the three functional domains are ~160 Å long. For colicin N, several helices in the channel-forming domain are postulated to be recruited into a periplasm-spanning role. On an ironic note, TolC is named for the 'Tolerant to Colicin' phenotype.

Future directions; future surprises?

The structural biology of transport across the two membranes of Gram-negative bacteria presents both opportunity and challenge. Opportunities are clear, as is the case with nearly all integral membrane proteins, because only a small number of structures of bacterial proteins involved in transport have been determined. Outer membrane porins, and, notably, the siderophore transporters FhuA and FepA are the only known structures to date of proteins involved in transport across the outer membrane. The TolC structure provides one image and indicates one mechanism for transport across the periplasm. No structures of inner membrane proteins involved in the formation of a translocase or that act as transporters have been solved at moderate or higher resolution. Another challenge is to come up with strategies to stabilize the open conformations of molecules such as TolC in order to obtain moderate- to high-resolution images of the two end states of a transport cycle. It may then be possible to utilize other experimental or computational methods to map out the conformational changes between these two states. Also, a unique challenge to elucidation of these systems at a molecular level is the development of methods to form complexes of proteins *in vitro* that approximate the two membranes found *in vivo*. Such preparations would be very useful for biochemical and biophysical characterization, as well as for crystallization. A low-resolution electron crystallographic structure of a gap junction channel [18] was obtained from just such a preparation; the membrane-spanning portions of two apposed hemichannels are seen in the electron density. Although that assembly is symmetrical with respect to the two identical membranes that gap junctions couple, this is unlikely to be the case for complexes containing plasma (inner) and outer membrane proteins. However, such a structure is suggestive of what may be obtainable in the future. Certainly, there is much to be done, and future results are likely to yield additional structural surprises.

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